

Research Article



A HPTLC Method for the Identification of Potential Therapeutic Compound of Kaempferol from *Ficus amplissima* Smith

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ABSTRACT

To evaluate the phyto constituent's composition and HPTLC fingerprint sequence profile of the medicinally important plant *Ficus amplissima* Smith. (Moraceae). The preliminary qualitative phytochemical screening was done and the HPTLC fingerprint analyses also were carried out. The Toluene-acetone-formic acid (4.5: 4.5: 1) was employed as mobile phase. The phytochemical screening showed the presence of unknown phenolic and kaempferol phyto compounds. The HPTLC fingerprinting of the extracts showed several peaks with different R_f values. The methanol extracts leaf, bark and fruit samples showed 4, 8 and 6 peaks in 5L concentration in 10 μL. The HPTLC fingerprint profile is used in differentiation of the species from the adulterant and act as biochemical markers for this medicinally important plant of *Ficus amplissima* may used to pharma industry and plant systematic studies.

Keywords: *Ficus amplissima*, HPTLC, Kaempferol, Phenolics.

INTRODUCTION

Traditional medicine (TM) is due a revival. For millennia, people around the world have healed the sick with herbal or animal-derived remedies, handed down through generations. In Africa and Asia, 80 per cent of the population still uses traditional remedies rather than modern medicine for primary healthcare. And in developed nations, TM is rapidly gaining appeal. Estimates suggest up to 80 per cent of the population has tried a therapy such as acupuncture or homeopathy. And a survey conducted earlier this year found that 74 per cent of US medical students believe that Western medicine would benefit by integrating traditional or alternative therapies and practices. Phytochemical analysis of plants were used in folklore has yielded a number of compounds with various pharmacological activities. Plants which are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids etc., have been found to have several biological properties. So the use of and search for drugs and dietary supplement derived from plants have increased in recent years.¹ Standardization of the plant material is need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physico-chemical characters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardization of herbs and its formulations. The WHO has emphasized the need to ensure the quality of medicinal plant products by using modern controlled techniques and applying suitable standards.^{2,3} HPTLC is a simple, rapid and accurate method for analyzing plant material.⁴ HPTLC fingerprint has better resolution and estimation of active constituents is done with reasonable accuracy in a shorter time. The HPTLC method can be used for phytochemical profiling of plants and

quantification of compounds present in plants, with increasing demand for herbal products as medicines and cosmetics there is an urgent need for standardization of plant products.⁵ Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment to ITM (Indian Traditional Medicine) and TCHM (Chinese traditional herbal medicine). The optimized chromatographic finger print is not only an alternative analytical tool for authentication, but also an approach to express the various patterns of chemical ingredients distributed in the herbal drugs and to preserve such "database" for further multifaceted sustainable studies. HPTLC finger print analysis has become the most potent tool for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drug.⁶

Ficus amplissima Smith belongs to the family Moraceae, commonly known as kal-itchchi. It is widely distributed all over India, northern Australia and other parts of Asia. The species is endemic in India and is commonly occurs in foothills of Himalaya, Assam, Sikkim, Kerala, Tamil Nadu, Andhra Pradesh, Maharashtra. The ethnobotanical views on *Ficus amplissima* suggest that the bark is used as a major ingredient for preparation of Ayurvedic herbal medicines against diabetes which is reported in indigenous medicine (Herbal formulations) in India. Bark is boiled with salt and boiled rice water in equal proportion and blue warm solution is given in colic⁷ and also leaves were used for jaundice, fruit is used to cure mental deranged and the latex is applied on the wound.⁸ Mitra and Kapoor⁹ reported the pharmacognostical studies of *F. tsiela* Roxb. In addition to that *Ficus amplissima* bark has been used for the ailments of throat—haws up cheesy laumps.¹⁰ The fruits are edible and chewed for mouth ulcers⁷ and also fruit is used to



cure mental derangement.⁸ Earlier studies in our laboratories showed that methanol extract of bark possessed anti-diabetic and antioxidant activities on Streptozotocin induced diabetes rats.¹¹ In addition to that, acetone extract of fruit exhibited a strong antioxidant activity, nutritional capacity and also contains non-polar compounds like fatty acids (Myristic acid, n-Hexadecanoic acid), sterols (Ergost-5-en-3-ol, β -Stigmasterol) and triterpenes (Lupenone, Lupeol).¹²

MATERIALS AND METHODS

Collection and Identification of Plant Material

The fresh leaf, bark, fruit parts were collected during the month of October 2009 from Ramapuram village, Sathyamangalam, district of Erode, Tamil Nadu, India. The taxonomic identity of the plant was confirmed by Dr. A. Rajendran, and voucher specimen (No: 006147) was deposited at Botany Department Herbarium, Bharathiar University, Coimbatore, Tamil Nadu. The plant materials were washed under running tap water to remove the surface pollutants and the different parts of leaf, bark, fruits were separated mechanically. The separated parts were air dried under shade. The dried sample was powdered and used for further studies.

Thin Layer Chromatography of Methanolic extract

Methanolic extract of *Ficus amplissima* plant samples were subjected to thin layer chromatographic studies, to find out the probable number of compounds present in them.

Extraction & Test solution preparation

The given dried Plant materials (5gm) were extracted with Methanol in Soxhlet apparatus for 3hrs. Cooled, filtered the content and concentrated using Vacuum flash evaporator. Dissolved the content with 1ml Methanol and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis.¹³

Sample loading, Spot development, Photo-documentation and Scanning

1.5 μ l of the above test solution and 2 μ l of standard solutions were loaded as 6mm band length in the 4 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Flavonoid) and the plate was developed in the respective mobile phase up to 90mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254nm and UV366nm. The developed plate was sprayed with respective spray reagent (Flavonoid) and dried at 100°C in Hot air oven. The plate was photo documented at Day light and UV 366nm using Photo documentation (CAMAG REPROSTAR 3) chamber. Before derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was

done at UV 254nm. The Peak table, Peak display and Peak densitogram were noted.

HPTLC Study

HPTLC precoated, silica gel G 60 F25 (Merck, Germany) plates were used for application of sample. A small quantity of extract was dissolved in methanol and sample was applied in precoated plate with the help of Linomat IV applicator. Solvent system optimized for TLC study was chosen for HPTLC study.

The details of HPTLC were as follows

Plate	:	Aluminium plate precoated with silica gel GF254
Thickness	:	0.2 mm
Plate size	:	10 x 10 cms
Sample application	:	10 μ l
Solvent system	:	Toluene-acetone-formic acid (4.5: 4.5: 1)
Detection	:	U.V. (254,366 nm)
Instrument	:	CAMAG TLC Scanner 3 & LINOMAT-V

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the methanolic extract revealed the presence of chemical constituents like Steroids, Tannins, Flavonoids, Carbohydrates, Saponins, Amino acids and alkaloids which was confirmed by performing TLC separation technique and different spraying reagents (the data was not given). The use of medicinal plants as Herbal Drugs, extracts have significantly increased throughout the world in the recent decades. According to WHO 80% of world population uses herbal remedies to cure diseases. Taking into consideration the popularity of the medicinal plants, these traditional drugs should be evaluated in detail for their Pharmacognosy, Phytochemistry and Pharmacology. Such a standardization of traditional medicine is the step to tap the emerging export market for natural drugs. All these medicinally important traditional crude drugs and their extracts should be standardized for its quality, purity and safety by using techniques such as organoleptic, microscopical, physical, chemical, biological evaluation. Pharmacognostic study mainly involves correct identity of crude drug with the help of drug organoleptic (sensory characters), microscopical (histological characters), physical and preliminary chemical evaluation.¹⁴ Present study has given very important information about High Performance Liquid Chromatographic (HPTLC) determination showed presence of flavonoids like kaempferol in the acetone extract of FAF. Most of the secondary metabolites found from herbs and spices are commercially important and are being used as pharmaceutically active compounds. Especially, flavonoids and phenolic acids are the key groups of secondary metabolites and bioactive compounds in plants.¹⁵ They are also kind of natural products and

antioxidant substances capable of scavenging free superoxide radicals, anti-aging and reducing the risk of cancer. Secondary metabolites are chemicals produced by plants for defense mechanism; and their functions in growth, photosynthesis, reproduction and other primary processes are not known yet. Secondary chemicals are important stated while enumerate the plant uses by widely used in Asia.¹⁶ The statement has attested that flavonoids reduce blood-lipid and glucose of humans in prevailing research on drugs. The methanolic extract of *Ficus amplissima* having the potentiality to scavenge the free radical contains phenolics, flavonoids and terpenoids which was analyzed by using HPTLC. The

peak table, peak display and peak densitogram were noted. Blue-violet colored zones were detected from the chromatogram after derivatization which confirmed the presence of phenolics (Figure 4). Thus, the presence of flavanoids in the methanolic extract of the leaf, bark and fruit of the plant of *Ficus amplissima* was confirmed by HPTLC analysis. The extract was run along with the standard flavanoid compound and it was observed that the extract showed the presence of presence of phenolic and flavonoids and it was confirmed from the chromatogram after derivatization. The peak heights of the respective kaempferol standard and phenolic compounds were given in the Figure 1,2,3,4 and Table 1.

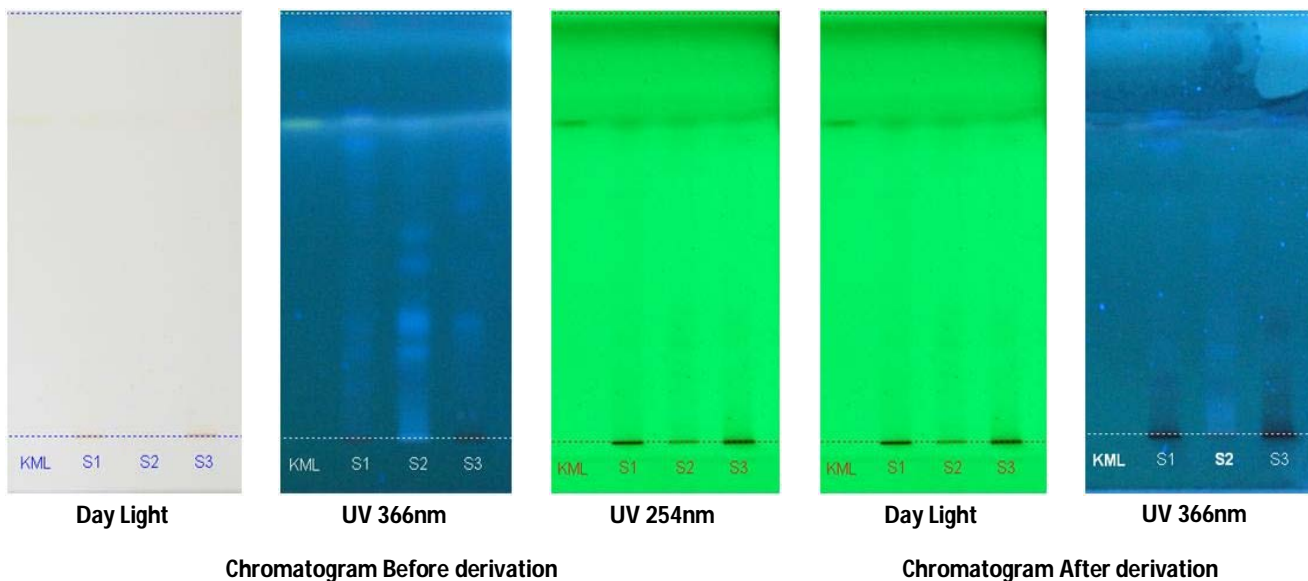


Figure 1: Track – Sample *Ficus amplissima* bark, leaf, fruit Methanolic extract Plant sample Peak display (Scanned at 254nm)

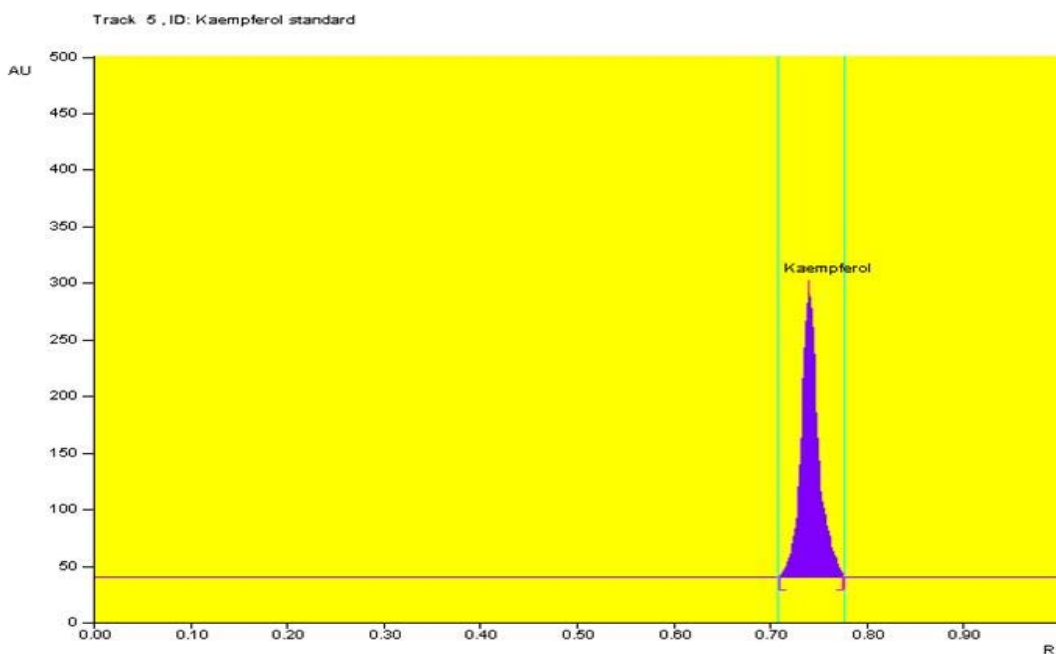


Figure 2: Track KML – Kaempferol standard Peak densitogram display (Scanned at 254nm)

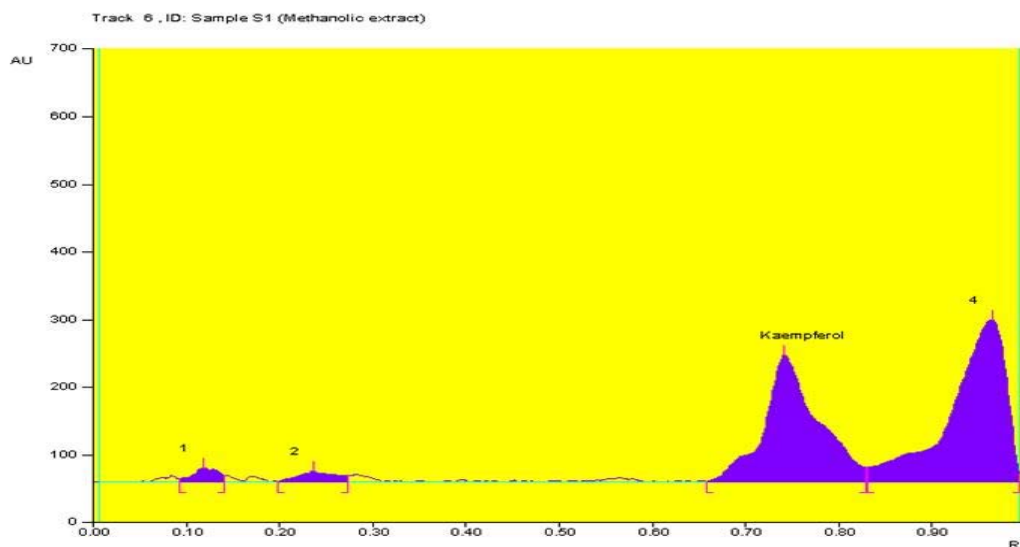


Figure 3: Track S1 – Bark Sample (S1) Methanolic extract plant sample Peak densitogram display (Scanned at 254nm)

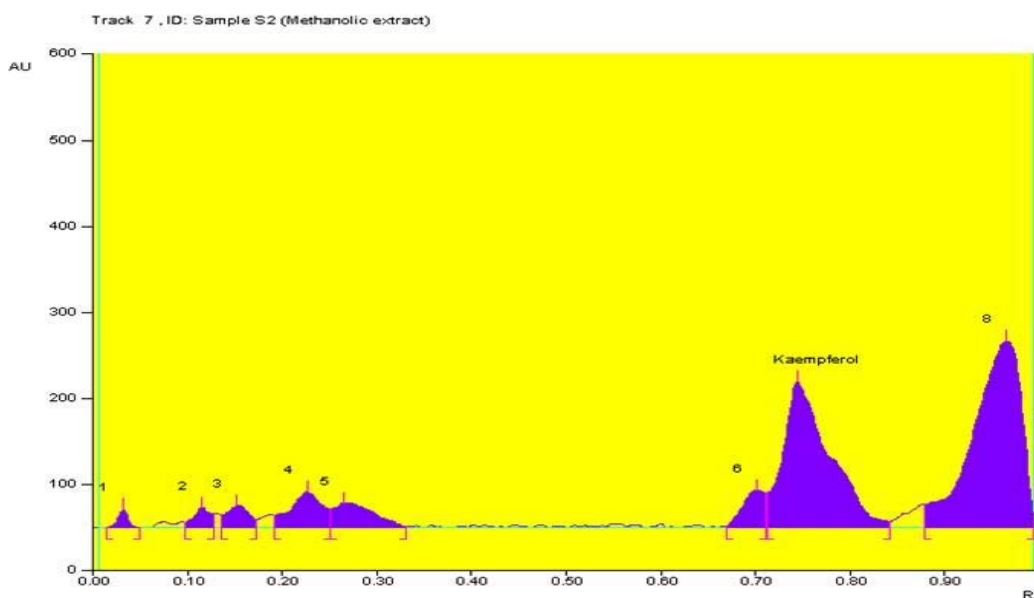


Figure 4: Track S2 – Leaf Sample (S2) Methanolic extract plant sample Peak densitogram display (Scanned at 254nm)

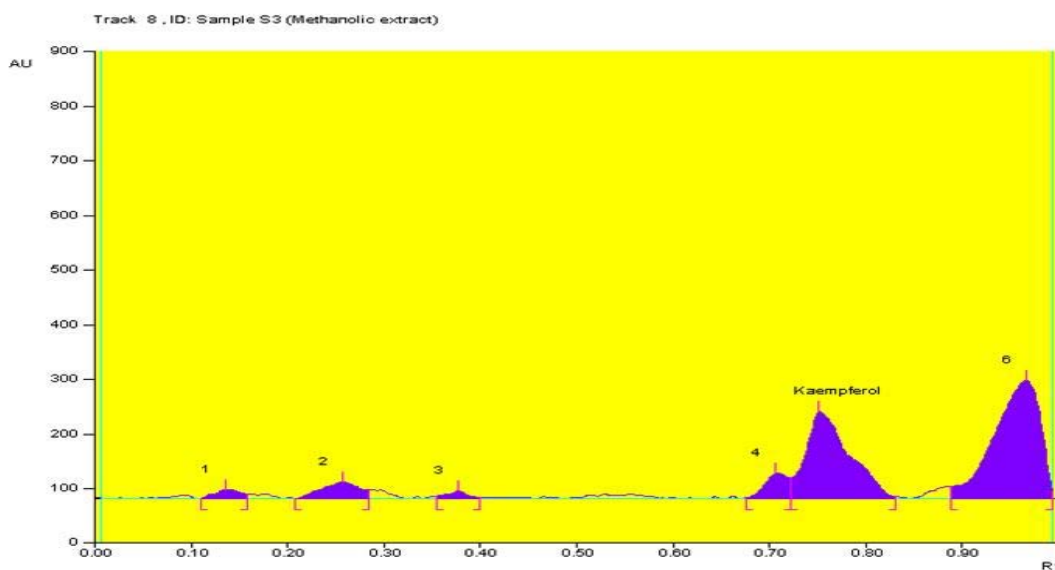


Figure 5: Track S3 – Fruit Sample (S3) Methanolic extract plant sample Peak densitogram display (Scanned at 254nm)

Table 1: Peak table

Track	Peak	Rf	Height	Area	Assigned substance
KML	1	0.74	315.2	11058.0	Kaempferol standard
Bark	1	0.12	20.6	533.8	Unknown
Bark	2	0.24	16.3	585.2	Phenolic 1
Bark	3	0.74	187.5	9958.3	Phenolic 2 (Kaempferol)
Bark	4	0.96	239.6	12911.6	Unknown
Leaf	1	0.03	21.1	208.0	Unknown
Leaf	2	0.12	23.5	383.0	Unknown
Leaf	3	0.15	25.4	568.3	Unknown
Leaf	4	0.23	41.2	1286.3	Unknown
Leaf	5	0.27	28.8	1127.8	Phenolic 1
Leaf	6	0.70	43.3	962.3	Phenolic 2
Leaf	7	0.74	169.4	7684.9	Phenolic 3 (Kaempferol)
Leaf	8	0.96	216.7	10080.6	Unknown
Fruit	1	0.14	16.9	430.7	Unknown
Fruit	2	0.26	30.7	1104.8	Phenolic 1
Fruit	3	0.38	13.9	275.8	Unknown
Fruit	4	0.71	45.7	1040.9	Phenolic 2
Fruit	5	0.75	158.9	6651.0	Phenolic 3 (Kaempferol)
Fruit	6	0.97	216.5	9519.7	Unknown

CONCLUSION

A rapid, easy, accurate and specific HPTLC method for quantitative estimation¹⁷ of kaempferol present in the *Ficus amplissima* plant parts of leaf, bark and fruit has been developed and validated. The data could be used as a quality control standard. The method used in this work resulted in good peak shape and enabled good resolution of kaempferol from other constituents of the plant material. Because recovery (98.33%) was close to 100%, there was no interference with the kaempferol peak from other constituents present in the plant.

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REFERENCES

- Uma B, Prabhakar K, Rajendran S, In vitro antimicrobial activity and Phytochemical analysis of *Ficus religiosa* and *Ficus bengalensis* L. against diarrhoeal enterotoxigenic E.coli. *Ethnobotanical leaflets*, 13, 2009, 472-474.
- Chaudhay Ranjit R, Herbal medicine for human health regional publication. SEARO, No.20, New Delhi, WHO, 1992, 1-80.
- WHO, Quality control method for medicinal plant material Geneva, WHO, 1998, 1-15.
- Wasim Aktar MD, Rajlakshmi Poi, Anjan Bhattacharya, Status of sennosides content in various Indian herbal formulations method standardization by HPTLC, Bangladesh J Pharmacol, 3, 2008, 64-68.
- Pawar RK, Sharma Shivani, Singh KC, Sharma Rajeev KV, Physico-chemical standardization and development HPTLC method for the determination of Andrographonin in Kalmgh Navyas Loha. An Ayurvedic formulation, Bangladesh J Pharmacol, 2, 2010, 295-301.
- Ram Mauji, Abdin MZ, Khan MA, Jha Prabhakar, HPTLC fingerprint analysis: A Quality control of Authentication of Herbal Phytochemicals, Verlag Berlin Heidelberg: Springer, 2011, 105.
- Pullaiah T, Medicinal plants in Andhra Pradesh, India, Regency publications, New Delhi. 2002, 130.
- Singh MP, Himadri panda FMA, Medicinal Herbs with their formulations, 2, 2005, 405.
- Mitra R, Kapoor LD, Pharmacognostical studies of *Ficus tsiela* Roxb, Indian J. Pharmacy, 34, 1972, 171.
- Pankaj Oudhia. Asi. Orlinseed (*Linum usitatissimum* L) and sanjeevani (*Sellaginella* bryophytes (L) Baker Based herbal formulations in Indian traditional healing for diabetes complications: Pankaj Oudhia's Ethnobotanical Survey from year 1990 – 2012, India, 7.
- Karuppusamy Arunachalam, Thangaraj Parimelazhgan, Antidiabetic and antioxidant efficacy of *Ficus amplissima* Smith. bark extract in Streptozotocin induced diabetic rats, J. Ethnopharmacol, 147, 2013, 302–310.
- Arunachalam K, Murugan R, Parimelazhagan T, Evaluation of antioxidant activity, nutritional and chemical composition of *Ficus amplissima* Smith. Fruit, International



- J. Food Properties, 2012, (ID: 642443 DOI:10.1080/10942912.2011.642443).
13. Egonstahl, A laboratory hand book of TLC, Springer Verlag, New York, 1990, 856, 878.
 14. Annie Felicia F, Muthulingam M, Phytochemical and HPTLC studies of methanolic extract of *Indigofera tinctoria* (Fabaceae), Int. J. of Pharm. & Life Sci, 3(5), 2012, 1670-1674.
 15. Kim D, Jeond S, Lee C, Antioxidant capacity of phenolic phytochemicals from various cultivars of plums, Food Chem, 81, 2003, 321-326.
 16. Bodeker G, Traditional health system: valuing biodiversity for human health and well being. In Cultural and Spiritual Values in Biodiversity, ed. D.A. Posey, Nairobi: Practical Action, 2000, 261– 284.
 17. Prasanthi NL, Ramarao N, High performance liquid chromatographic estimation of ziprasidone in pharmaceutical dosage forms, International J. Pharma. Pharmaceut. Sci, 2(1), 2010, 120-122.

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